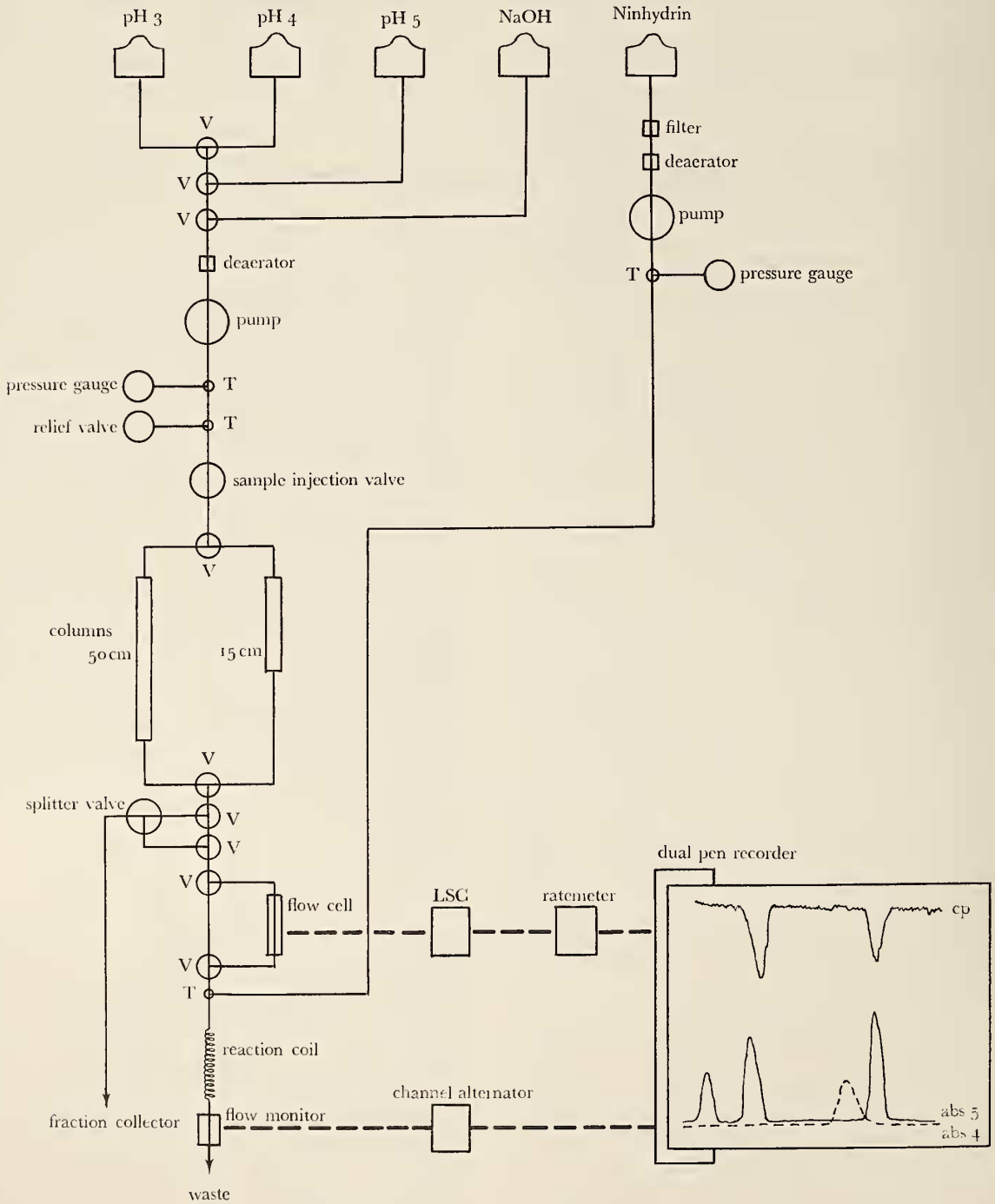

pH 3
pH 4
pH 5
NaOH
Ninhydrin
V
V
V
filter
deaerator
pump
T
pressure gauge
deaerator
pump
pressure gauge
T
relief valve
T
sample injection valve
V
columns
50 cm
15 cm
V
splitter valve
V
V
V
flow cell
LSC
ratemeter
dual pen recorder
cp
V
T
reaction coil
channel alternator
fraction collector
flow monitor
abs 5
abs 4
waste

mmole; New England Nuclear). The mussels were kept in a closed container connected to a vacuum such that oxygen was available yet CO_2 was trapped in a 1 M KOH solution. Samples of sea water and KOH (0.1 ml) were taken at 1, 2, 4, 8, 24, 48, and 72 hours. These were put in 10 ml of Bray's solution (Bray, 1960) and then counted on a Packard 3320 liquid scintillation spectrometer to determine label lost by leakage to sea water and label respired as $^{14}CO_2$.

After 72 hours, the soft tissue of each mussel was removed from its shell, weighed (1.25, 1.25, 1.58 g respectively for mussel A, B and C) and homogenized with a Virtis blender. Extraction of the protein fraction followed the method of Shibko *et al* (1967). Two separate hydrolysates were performed on the dried protein, one employing 4% thioglycolic acid in 6 N HCl to protect tryptophan (Matsubara & Sasaki, 1969) and a second utilizing performic acid to enable half-cystine to be determined as cysteic acid (Blackburn, 1968). Each hydrolysate was evaporated on a rotary evaporator and diluted with a known concentration of norleucine and α amino β guanido propionic acid, which served as internal standards.

Three amino acid chromatographs were done for mussels A and B, two for mussel C. The first quantified the acidic and neutral amino acids, the second the basic amino acids and the third determined cysteic acid. A cysteic acid determination was not performed on mussel C. The amino acids were quantified with an in-line amino acid analyzer-liquid scintillation flow monitor (Piez, 1962) as diagrammed in Figure 1. Columns of 55 × 0.9 cm and 10 × 0.9 cm packed with Aminex A5 (BioRad Laboratories) were used for the separation of acidic-neutral and basic amino acids. Buffer flow rate was 40 ml per hour; ninhydrin 20 ml per hour. Buffers and ninhydrin reagent were prepared as described by Benson & Patterson (1971). The micromoles of each amino acid/100 mg dry protein were calculated using the internal standards norleucine (acid and neutral amino acids) and α amino β guanido propionic acid (basic amino acids). The specific activities of each amino acid were also calculated, the calibration being performed by chromatographing a known activity of glycine.

(← on facing page)

Figure 1

Amino acid analyzer-liquid scintillation flow monitor system. Dual pen recorder – Honeywell Electronik 194, ratemeter – Packard 280A, LSC – liquid scintillation counter (Packard 3320) fitted with 1 ml lucite flow cell packed with anthracene, channel alternator – ISCO 1130, flow monitor – ISCO UA 4, fraction collector – ISCO 567, columns 50 cm and 15 cm – Cheminert PRV 500, pressure gauge – Cheminert PG200 (ninhydrin line) and PG1000 (buffer line), pump – Milton Roy Instrument Mini-pump (0 to 160 ml/hr), V – Cheminert CAV3031 three-way valve, and T – Cheminert CJ3031 tee.

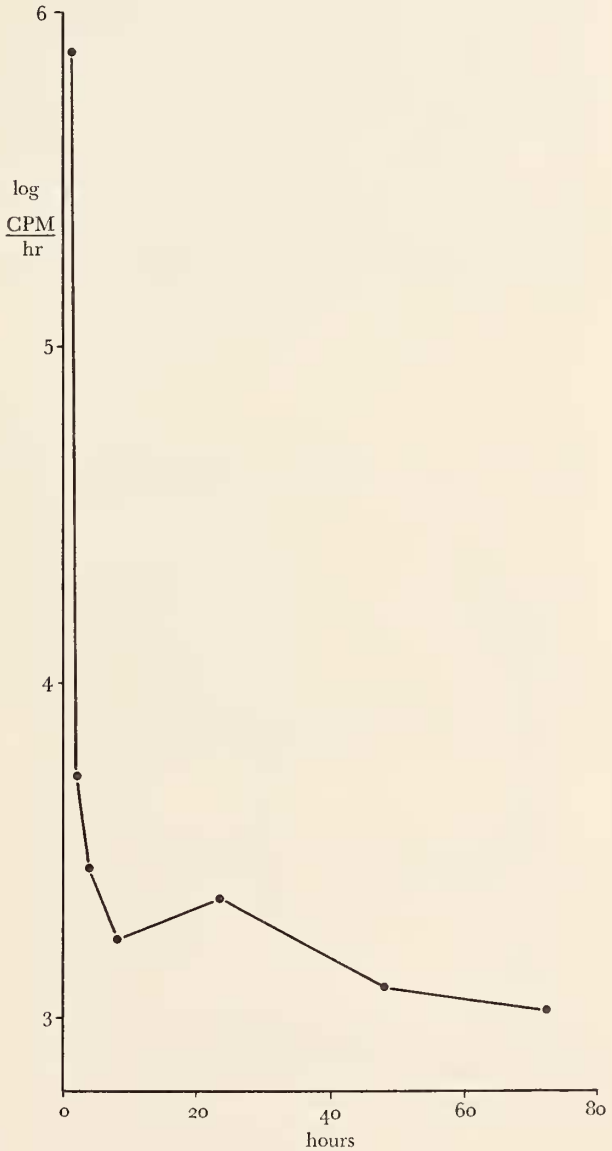


Figure 2

Radioactivity detected in incubation sea water for mussel B after injection of 50 Ci of (U-^{14}C) glucose. Ordinate: $\log_{10}$ cpm/hr (uncorrected for quenching) incorporated into each fraction averaged over periods indicated by abscissa.

RESULTS AND DISCUSSION

A plot of sea water activity against time (Figure 2) shows substantial loss of activity into the sea water during the first hour after injection, presumably due to leakage of (U-^{14}C) glucose. For subsequent samples, the activity rapidly dropped and attained a constant low level throughout the sampling period, indicating continued leakage or excretion of labelled products.

The concentration and specific activities of each amino acid in whole body protein are presented in Table 1. Aspartic acid, glutamic acid and alanine showed the highest concentrations in all 3 mussels while tryptophan showed the lowest. There is some variation in the absolute amount of each amino acid, and mussels B and C match much more closely than either does with A. The relative amounts are similar in each individual, so this anomaly is best explained as an inconsistency in the internal standard.

The specific activities indicate that aspartic acid, glutamic acid, alanine, serine, half-cystine and glycine incorporated label. These are therefore inferred to be nonessential. The first three showed consistently high specific activities, probably due to biosynthetic routes involving transamination from intermediates of glycolysis and the Krebs cycle (MAHLER & CORDES, 1966).

No label was incorporated into threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, lysine, histidine, or arginine. These are therefore inferred to be essential. No label was incorporated into tyrosine, but it has been demonstrated in mammals (MEISTER, 1965) and crustaceans (ZANDEE, 1966) that a synthetic pathway exists from phenylalanine. Since this pathway was not investigated, the essentiality of tyrosine is unresolved. No label was incorporated into proline in mussels A and B when (U-^{14}C) glucose was injected. Mussel C was injected with (U-^{14}C) glutamic acid, a more immediate

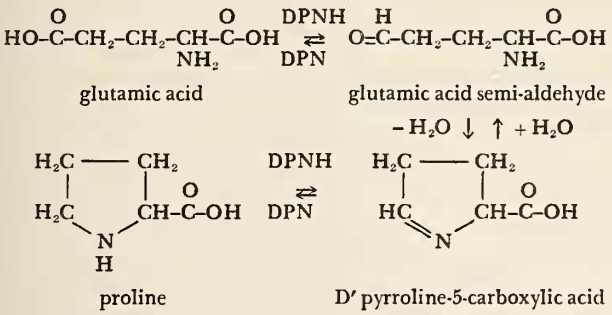
Table 1

Specific activities of amino acids and composition of whole body proteins.

Amino acid	Mussel A Conc.	Mussel A Spec. Act.	Mussel B Conc.	Mussel B Spec. Act.	Mussel C Conc.	Mussel C Spec. Act.	Essential
Half-cystine	4.8	3050	17.0	0	n.d.	n.d.	−
Aspartic Acid	139	2230	192	1210	171	1430	−
Threonine	21.8	0	88.8	0	77.5	0	+
Serine	23.6	3290	91.6	460	75.7	0	−
Glutamic Acid	61.1	616	194	756	153	348	−
Proline	12.5	0	20.4	0	22.0	0	+
Glycine	33.5	656	92	172	92.8	0	−
Alanine	39.5	2500	112	1340	103	418	−
Valine	21.9	0	65.2	0	58.8	0	+
Methionine	8.8	0	24.8	0	26.5	0	+
Isoleucine	18.2	0	47.3	0	50.8	0	+
Leucine	35.9	0	90.7	0	79.5	0	+
Tyrosine	13.6	0	35.9	0	38.5	0	?[1]
Phenylalanine	12.9	0	34.4	0	35.6	0	+
Tryptophan	1.3	0	2.8	0	3.0	0	+
Lysine	27.0	0	50.0	0	84.5	0	+
Histidine	6.3	0	12.6	0	19.6	0	+
Arginine	29.2	0	51.4	0	71.3	0	+

Concentration is in micromoles/100 mg protein
Specific activity is in cpm/micromole
n.d. = not determined
[1] possibility of biosynthesis from phenylalanine not investigated

precursor of proline, to see if the pathway for proline synthesis exists (MAHLER & CORDES, 1966):



No label was incorporated so it must be inferred that proline is an essential amino acid.

With the exception of a requirement for proline, juvenile California mussels require the same amino acids as growing rats (MEISTER, 1965), the shrimp *Palaemon serratus* (COWEY & FORSTER, 1971), the red abalone *Haliotis rufescens* (Allen and Kilgore, in press) and the crab *Cancer magister* (G. Lasser, personal communication). It differs in amino acid requirements from man only in the requirement for arginine (MEISTER, 1965). It is indeed probable that there is little deviation from this basic pattern anywhere in the animal kingdom.

ACKNOWLEDGMENTS

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Growth in the Black Abalone, *Haliotis cracherodii*

BY

MARY BERGEN WRIGHT[1]

(4 Text figures)

INTRODUCTION

GROWTH RATE is one of the fundamental parameters that must be determined if a population is to be properly managed. If the growth rate is known, then other parameters such as the age at maturity and death are known. However, at the present time, there is little information on growth rates in marine invertebrates, even in commercially important species such as the black abalone, *Haliotis cracherodii* Leach 1817. Cox (1962) stated that, in October 1955, John Fitch found two size classes of small abalones, one ranging from 1.0–8.5 mm and the other from 17.0–35.0 mm. Cox suggested that the smaller was the result of the summer spawn of 1955 and the larger the result of the previous year's spawn. LEIGHTON & BOOLOOTIAN (1963) measured growth in black abalones at Palos Verdes and Point Dume, California. To do this, they removed the abalones from the rocks for marking and measuring. However, in the present study the abalones were marked and measured *in situ* so as not to injure them or disturb their normal behavior, and thus interfere with their growth.

MATERIALS AND METHODS

Abalones were marked and measured throughout the rocky intertidal area on the north side of Christy Beach on Santa Cruz Island, 42 km south of Santa Barbara, California. The area is composed of broken basaltic rock.

Abalones were tagged *in situ* in three ways: (1) small numbered tags were glued to the clean, dry shell with Dekophane adhesive; (2) monofilament nylon line with color-coded beads was threaded through the respiratory holes; and (3) numbers were glued to pieces of acetate and these were attached to the shell with cement. None of the methods was entirely satisfactory. Dekophane glue could only be applied when the shell was dry and when there was sufficient time for the glue to set before the tide came in. Even when the glue set properly, snails and limpets often grazed the numbers off in a month or two. Beads could only be used when the respiratory holes could be reached and often eroded away or broke, leaving the monofilament. However, as long as large numbers of abalones were marked, the methods were satisfactory for experiments lasting up to a year.

Growth was measured by filing a cross in the apex of

Table 1

Measurements taken to Estimate Measurement Error

Measurement 1	Measurement 2	Difference	Measurement 1	Measurement 2	Difference
38.0	37.5	0.5	126.0	125.0	1.0
39.5	40.0	0.5	126.5	126.0	0.5
48.0	49.5	1.5	126.5	127.0	0.5
72.0	72.0	0	128.0	128.5	0.5
77.0	77.0	0	129.5	129.0	0.5
80.0	78.5	1.5	129.5	129.0	0.5
88.0	88.5	0.5	129.5	128.5	1.0
89.0	89.0	0	130.0	128.0	2.0
90.0	90.5	0.5	131.0	129.5	1.5
100.5	101.0	0.5	131.0	131.0	0
101.0	101.5	0.5	131.5	129.5	2.0
109.0	108.0	1.0	132.0	131.5	0.5
109.5	109.0	0.5	132.0	132.5	0.5
113.0	113.0	0	133.0	133.0	0
114.0	113.5	0.5	135.0	135.5	0.5
114.0	114.0	0	135.5	135.5	0
116.0	117.0	1.0	136.0	134.5	1.5
117.0	116.5	0.5	137.5	136.0	1.5
117.0	116.5	0.5	141.0	141.0	0
120.0	120.0	0	141.0	141.0	0
121.0	120.5	0.5	141.0	141.0	0
121.5	122.5	1.0	143.0	142.5	0.5
125.0	125.5	0.5	143.5	143.0	0.5
			148.5	149.0	0.5

$N = 47$
$\bar{X} = 0.60$

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the shell and measuring the maximum distance between the cross and the anterior edge of the shell with dividers. The cross provided a constant reference point from which to measure and thus reduced measurement error. Each time the study site was visited, "new" abalones were marked and the previously marked animals were measured. Some individuals were measured for more than two years; others immediately disappeared or, more commonly, lost their tags. The data for 74 animals that were measured for two months or more are presented below.

To determine the measurement error, on several occasions each abalone was measured and then remeasured later in the day (Table 1). These successive measurements varied at most 2 mm.

RESULTS

Figure 1 records growth for each individual measured as:

$$\frac{365\ (\text{final size}—\text{initial size})}{\#\ \text{days between measurements}} \pm \frac{365\ (2\,\text{mm})}{\#\ \text{days between measurements}}$$

The "confidence interval" indicates the range of variation in growth rate due to measurement error. If the "confidence interval" crosses the zero line, there was no significant change in size. Small abalones grew faster than the

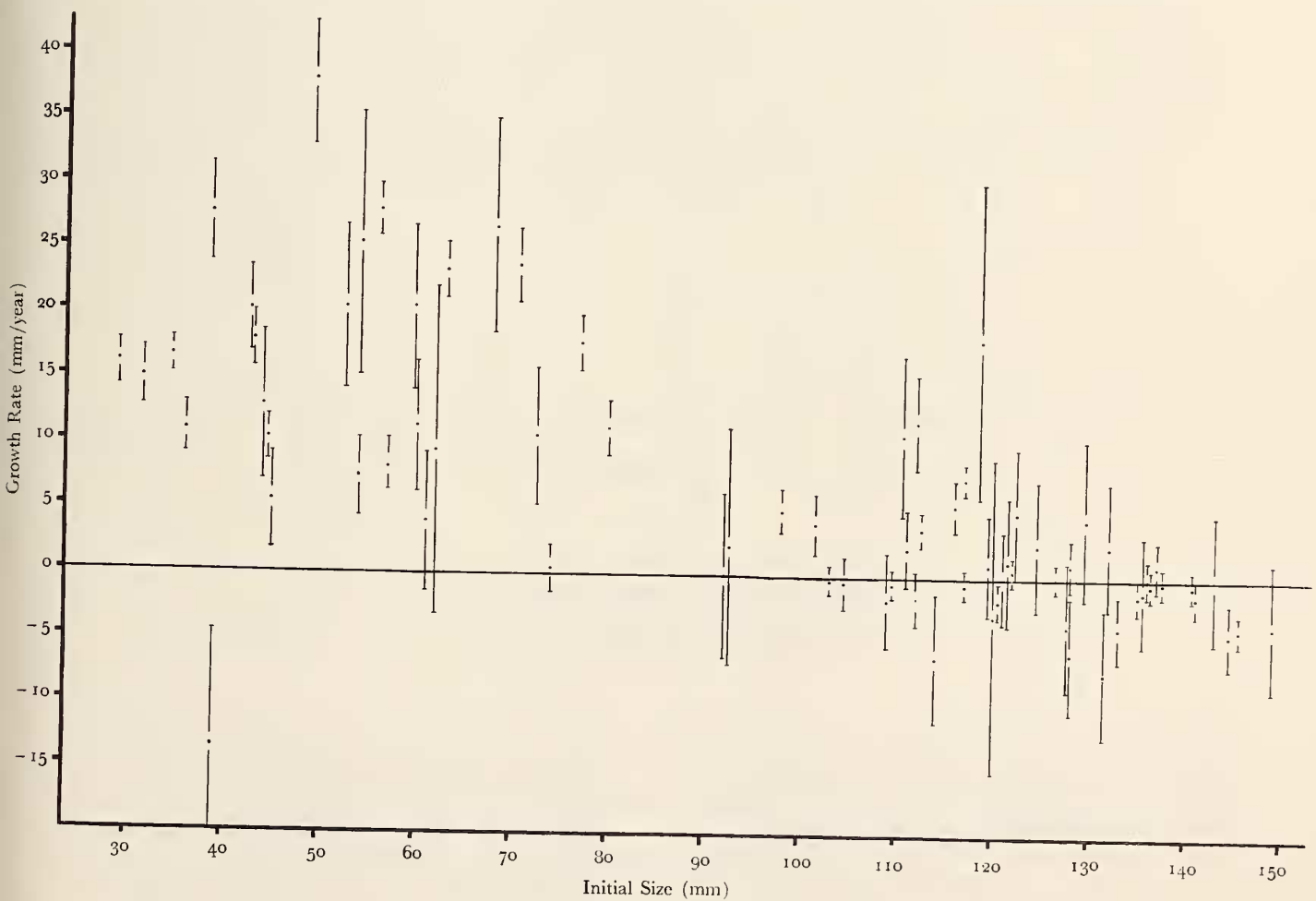

Figure 1

Growth rate for each abalone measured for two or more months